# Evaluation of coated steroidal implants containing trenbolone acetate and estradiol-17β on live performance, carcass traits, and sera metabolites in finishing steers

Zachary K. Smith\*, Alex J. Thompson\*, John P. Hutcheson<sup>†</sup>, Wade T. Nichols<sup>†</sup>, and Bradley J. Johnson<sup>\*,1</sup>

\*Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409; †Merck Animal Health, Madison, NJ 07940

**ABSTRACT:** Crossbred beef steers (n = 240; 12) pens/treatment; initial BW =  $305 \pm 17.7$  kg) were used in a randomized block design feedlot study to evaluate the influence of coated trenbolone acetate (TBA) and estradiol-17 $\beta$  (E<sub>2</sub>) implants (Merck Animal Health, Madison, NJ) on gain performance, carcass traits, and sera metabolites. The five treatments were no implant (NI), Revalor-XR on d 0 [200 mg TBA + 20 mg  $E_2$  (coated); XR], Revalor-XS on d 0 [200 mg TBA +  $40 \text{ mg E}_{2}$  (total):  $80 \text{ mg TBA} + 16 \text{ mg E}_{2}$  (noncoated) and 120 mgTBA + 24 mg  $E_2$  (coated); XS], Revalor-200 on d  $0 [200 \text{ mg TBA} + 20 \text{ mg E}_2 \text{ (noncoated); E200], or}$ Revalor-200 on d 70 (D200). Interim BW and blood were collected on d 0, 14, 35, 70, 105, 140, and 175 prior to feeding and on d 213 prior to shipping. Following a 24 h clot at 4 °C, sera was harvested to quantify circulating E<sub>2</sub>, IGF-I, NEFA, serum urea-N (SUN), and  $17\beta$ -trenbolone ( $17\beta$ -TbOH). Implanted steers had greater ( $P \le 0.05$ ) ADG, G:F, and final BW than NI controls. Implants increased (*P* < 0.05) HCW by 8%, 366 vs. 391, 414, 380, and  $396 \pm 6.4$  kg, for NI vs. XR, XS, E200, and D200, respectively. The greatest ( $P \le 0.05$ ) dressing percentage, yield grade, and calculated empty body fat occurred in XS, which had greater (P < 0.05) rib fat than NI, XR, and D200. Marbling scores in NI were greater (P < 0.05) than E200 and D200; steers in XR and XS were intermediate (P > 0.10), not differing from NI, E200, or D200. An implant × day interaction ( $P \le 0.01$ ) was noted for circulating E<sub>2</sub>, IGF-I, SUN, and 17β-TbOH. Implanted steers had elevated ( $P \le 0.05$ ) sera E<sub>2</sub>, IGF-I, and 17 $\beta$ -TbOH, and decreased (P < 0.05) SUN following implantation compared to NI controls. Serum NEFA differed (P < 0.01) over time, but did not differ (P > 0.10) due to implant treatment. These data indicated that the polymer coating applied to the XR implant delayed release of steroidal hormones congruently to D200, with no negative impact on marbling. The greatest dose of  $E_{2}$ , contained in XS, provided improvements in gain and carcass weight without detriment to marbling scores compared to NI.

Key words: beef, estradiol-17 $\beta$ , IGF-I, implant, steer, trenbolone acetate

 $\bigcirc$  The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

> J. Anim. Sci. 2018.96:1704–1723 doi: 10.1093/jas/sky095

<sup>1</sup>Corresponding author: bradley.johnson@ttu.edu

Received January 9, 2018. Accepted March 7, 2018.

# INTRODUCTION

Anabolic implants containing trenbolone acetate (**TBA**) and estradiol-17 $\beta$  (**E**<sub>2</sub>) have been approved for use in confined finishing cattle by

Supported in part by funding from Merck Animal Health, Madison, NJ, and the Gordon W. Davis Regents Chair in Meat Science and Muscle Biology Endowment at Texas Tech University, Lubbock.

the U.S. Food and Drug Administration (FDA) for over 25 y. Implants increase average daily gain (ADG), gain to feed (G:F), and decrease marbling and yield grade compared to nonimplanted cattle fed for slaughter at equal days on feed. Implants increase frame size and delay fattening. This shift in frame size, requires implanted cattle be fed to greater final shrunk body weight (FSBW) in order to reach similar empty body fat (EBF) percentage as compared to nonimplanted cattle. Currently, no other technology is available to producers that match the improvements in performance and hot carcass weight (HCW) achieved via implants at equal back fat (BF) (Reinhardt, 2007).

Effective anabolic payout in noncoated TBA + E, implant products is 60 to 120 d (Mader, 1998). More commonly, cattle feeders have begun placing lighter weight cattle on feed and this younger animal spends greater than 200 d in the feedlot. In the last decade, the FDA has approved four coated implants that extend hormonal payout in excess of 200 d postimplantation for use in feedlot cattle. When a noncoated implant is administered, the initial 40 d period is when carcass protein gain is maximal (Johnson et al., 1996a). Johnson et al. (1996a) reported that steers implanted with 120 mg TBA + 24 mg  $E_2$  had a rapid increase in trenbolone-17 $\beta$  (17 $\beta$ -TbOH) and E, by d 2 following implantation. During the initial 40 d, an increase in circulating concentrations of sera IGF-I for implanted steers over controls also occurred (Johnson et al., 1996b). Parr et al. (2014) also detected increased circulating concentrations of IGF-I in steers implanted with a 120 mg TBA + 24 mg  $E_2$  (noncoated) or 80 mg TBA + 16 mg  $E_{2}$  (noncoated portion of Revalor-XS, Merck Animal Health, Madison, NJ) by d 27. The primary objective of this experiment was to compare coated implants to noncoated implants in steers fed for 213 d.

# MATERIALS AND METHODS

# Use of Animal Subjects

The Texas Tech University Animal Care and Use Committee (ACUC) approved all procedures involving the use of animals in this study (ACUC number 16029-04). The experiment was conducted at the Texas Tech University Burnett Feedlot Research Center (TTUBFRC) that is located approximately 11 km east of New Deal, TX.

#### Initial Processing

Two-hundred and sixty-six British × Continental crossbred yearling steers were received in three full and one half load truckload lots over the course of a 2-week period in late May and early June of 2016. Upon arrival, all steers were placed in dirt surface receiving pens and provided ad libitum access to water, long stem grass hay, and offered a 65% concentrate (DM basis) starter ration at 1% of BW. Within 3 d of arrival to the TTUBFRC, each truckload of steers was processed and all steers were individually weighed on a legal for trade scale certified by the Texas Department of Agriculture (readability  $\pm$  0.454 kg). Initial processing also included the following procedures: application of an unique individual ear tag in duplicate, vaccination against: Infectious Bovine Rhinotracheitis, Bovine Viral Diarrhea Types I and II, Bovine Parainfluenza-3, and Bovine Respiratory Syncytial Virus (Bovi-Shield Gold 5; Zoetis, Florham Park, NJ), clostridial species (Vision 7; Merck Animal Health, Madison, NJ), and mycoplasma bovis (Myco-Vac B; Texas Vet Lab Inc., San Angelo, TX) diseases. Whereas, parasite control was provided via administration of fenbendazole (Safeguard; Merck Animal Health) and ivermectin (Ivermectin pour-on; Vet One, Boise, ID) for internal and external parasites according to label instructions.

#### **Experimental Design and Treatments**

Five treatments were used in a randomized complete block design feedlot study. Implant treatments included:

- 1) Negative control given no implant (NI).
- Revalor-XR administered subcutaneously in the center one-third of the ear on d 0 [200 mg TBA + 20 mg E<sub>2</sub> (coated, polymer coating is proposed to degrade entirely by d 70 following implant administration); XR].
- Revalor-XS administered subcutaneously in the center one-third of the ear on d 0 [200 mg TBA + 40 mg E<sub>2</sub> (total): 80 mg TBA + 16 mg E<sub>2</sub> (non-coated) and 120 mg TBA + 24 mg E<sub>2</sub> (coated, polymer coating is proposed to degrade entirely by d 70 following implant administration); XS].
- 4) Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 [200 mg TBA + 20 mg E<sub>2</sub> (noncoated); E200]
- 5) Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 during interim BW measurement collection (**D200**).

# Study Initiation

Three individual BW measurements were captured on d = 2, -1, and 0 relative to treatment initiation. The d -2 BW measure was used to exclude steers with abnormal health, extreme BW, or obvious temperament issues from consideration for study enrollment. On d -1, steers (n = 240) that were selected from the larger population of steers on the basis of d -2 BW measurements were divided into 12 blocks by BW and returned to 12 dirt surfaced pens. Steers within each block were assigned to pen based on the average of the consecutive BW measurements taken on d - 2 and -1 in a method that reduced average pen BW variability between pens within block. Treatments (n = 5) were randomly assigned to pen within block. Upon trial initiation (d 0), steers were individually weighed and sorted into 60 concrete, slatted-floor pens (4 steers/pen;  $2.9 \times 5.5$  m with 2.4 m of linear bunk space). The respective implant treatments were administered by the same individual blinded to actual implant treatment to the appropriate steers on d 0 (this occurred on d 70 for D200 steers), and all cattle were fed to provide ad libitum access to feed once daily at 0800 h for the duration of the experiment. Individual BW measurements captured on d-1and 0 were averaged and used as the initial trial BW (un-shrunk initial average BW=  $310 \pm 18.0$  kg). Implants checks for implant retention occurred on d 35 and 70 for XR, XS, and E200 steers, and d 105 for D200 steers.

# Diet

Steers were gradually acclimated from the 65% concentrate diet to the final diet using a 4-step process (65, 75, 85, and 90% concentrate diets). At treatment initiation, all steers were consuming the 75% concentrate diet. By d 11, posttreatment initiation steers were consuming the 90% concentrate diet (Table 1). The diets were formulated to meet nutrient requirements (NRC, 1996) for growing-finishing beef cattle and were prepared in the Texas Tech Burnett Center Feed Mill. The final diet contained (DM basis): 64.6% steam-flaked corn, 20.1% wet corn gluten feed, 7.9% alfalfa hay, 3.9% mineral and vitamin supplement, 3.0% fat, and 0.50% urea. The final diet contained  $13.3 \pm 0.36$  % CP, and was calculated to provided 0.97 and 0.66 Mcal/0.454 kg of  $NE_m$  and  $NE_g$ , respectively. The final diet was also formulated to supply on a DM basis: monensin sodium at 33.0 mg/kg (Rumensin 90, Elanco Animal Health, Greenfield, IN) and

**Table 1.** Ingredient formulation and analyzedchemical composition of Finishing Diet<sup>1</sup>

Item	Value	SD
Ingredient, % DM		
Steam-flaked corn	64.56	
Wet corn gluten feed	20.07	
Alfalfa hay	7.93	
Fat (yellow grease)	3.07	
Supplement <sup>2</sup>	1.99	
Calcium Carbonate	1.87	
Urea	0.51	
Analyzed composition <sup>3</sup>		
Diet DM, %	78.32	1.315
СР, %	13.27	0.356
ADF, %	8.38	0.426
NDF, %	16.60	1.394
ASH, %	4.48	0.414
NE <sub>M</sub> , Mcal/0.454 kg <sup>4</sup>	0.97	
$NE_{G}$ , Mcal/0.454 kg <sup>4</sup>	0.66	

<sup>1</sup>All values except Diet DM on a dry matter basis.

<sup>2</sup>Supplement composition (DM basis): 67.755% Cottonseed meal, 15.000% NaCl, 10.000% KCl, 3.760% Urea, 0.986% Zinc sulfate, 0.750% Rumensin-90 (Elanco, Greenfield, IN), 0.506 Tylan-40 (Elanco), 0.500% Endox (Kemin Industries, Des Moines, IA), 0.196% Copper sulfate, 0.167% Manganese oxide, 0.157% vitamin E (500 IU/g), 0.125% selenium premix (0.2% Se), 0.083% iron sulfate, 0.010% vitamin A (1,000,000 IU/g), 0.003% ethylenediamine dihydroiodide, and 0.002% cobalt carbonate.

<sup>3</sup>Composition from 6 samples that were composited by interim weigh period from weekly diet samples and analyzed at a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Diet DM determined weekly (forced-air oven for 24 h at 100 °C).

<sup>4</sup>Tabular values.

tylosin phosphate at 11.0 mg/kg (Tylan 40, Elanco Animal Health).

#### Intake Management

Feed bunks were evaluated at approximately 0730 h daily to estimate orts and adjust feed calls to ensure *ad libitum* access to feed. The bunk management approach was to achieve less than 0.454 kg of dry orts in the bunk at the time of feeding each day. Diets were mixed in a paddle type mixer, transferred by drag chain conveyor to a tractor pulled mixer (Rotomix 84–8 wagon mixer; Rotomix, Dodge City, KS; scale readability  $\pm$  0.454 kg), and delivered once daily beginning at 0800 h.

The total mixed ration was sampled weekly throughout the course of the study and split into 2 aliquots. One aliquot of the weekly sample was immediately taken and dried in duplicate in a forced-air oven at 100 °C for 24 h in order to determine DM content of the diet, which was then utilized to determine total DMI for each week. At the conclusion of the study the second aliquot of the weekly diet samples, were composited by 35 d period for chemical analyses of CP, ADF, NDF, and ash content (Dairy One Forage Laboratory, Ithaca, NY) using AOAC procedures. Diet composition and chemical analysis of the 90% concentrate diet can be found in Table 1.

Orts were collected, weighed, and dried in a forced-air oven at 100 °C for 24 h in order to determine DM content if carryover feed went out of condition, or was present on weigh days. If carryover feed was present on weigh days, the residual feed was removed prior to the collection of BW measurements. The DMI of each pen was adjusted to reflect the total DM delivered to each pen after subtracting the quantity of dry orts for each interim period.

# **Production Data**

Individual BW measurements were taken before feed delivery (0630 h) on d -2, -1, 0, 14, 35, 70, 105, 140, and 175 relative to trial initiation, and on d 213 prior to shipping. There was no restriction of feed or water prior to weighing steers. Cumulative performance was calculated using the average of the d -1 and d 0 BW shrunk 1.5% as the initial on test BW, and then by assigning a 1.5% shrink to the final BW on d 213, and by also assuming a common dressing percentage among treatments of 62.50%. Interim period live performance was also tabulated and analyzed without applying any shrink to initial BW, d 70 BW, d 140 BW, or d 213 BW. Gain efficiency (G:F) was calculated as: (ADG / DMI). The energy density of live weight gain (GED) was calculated as: (Energy retained, Mcal/d)/(ADG, kg (d), where (*Energy retained*, Mcal / d) = Feed available for gain,  $kg(FFG) \times Diet NEg(MCal/kg)$ , and FFG = (DMI - DMI for maintenance), and DMI formaintenance =  $((0.077 \times BW^{0.75}) / (Diet NEm Mcal)$ (kg)). The units for GED are in (Mcal/kg) which assigns an arbitrary number in order to determine and compare the caloric content of live weight gain among treatment groups.

#### Sera Metabolites

Whole blood was collected and then harvested as sera on d 0, 14, 35, 70, 105, 140, 175, and 213 relative to trial initiation. Sentinel steers (n = 2 steers/ pen; 24 steers/treatment) identified prior to the initiation of the study were used for sera metabolite determinations. The Sentinel steers were selected from each pen based on the average of d -2 and -1 BW measurements. The 2 steers/pen with an average d -2 and -1 BW closest to the mean BW of their home pen were selected for blood collection. Whole blood was collected into 15 mL nonadditive evacuated tubes and allowed to clot for 24 h at 4 °C and subsequently centrifuged at 1250 × g at 4 °C in order to harvest sera. This sera was subsequently used to quantify circulating concentrations of E<sub>2</sub>, IGF-I, NEFA, serum urea-N (SUN), and 17β-TbOH.

Circulating  $E_2$  concentration was determined via RIA procedures using methods described by (Kirby et al., 1997). The  $E_2$  assay was analyzed using sera pooled by pen and day. Cross-reactivity of the antibody used were 100% for  $E_2$ , 6.5% for estriol, 5.2% for estradiol-17 $\alpha$ , 0.6% for estrone, and < 0.01% for aldosterone, androstenedione, cholesterol, progesterone, and testosterone (Kirby et al., 1997). The intra- and inter-assay coefficients of variation for the estradiol-17 $\beta$  assay were 9.25% and 3.91%, respectively, and assay sensitivity was 0.5 pg/mL.

Circulating IGF-I concentration was quantified via ELISA procedures (Quantikine Human IGF-I ELISA, R & D Systems, Minneapolis, MN). The IGF-I assay was analyzed using sera pooled by pen and day. Samples were assayed according to the manufacturers' recommendations. Prior to analysis raw sera samples were extracted in order to reduce IGF binding protein interference. The standard curve constructed for the IGF-I assay was between 9.4 and 600.0 ng/mL. For the IGF-I analysis the intra-assay CV was 6.7% and the inter-assay CV was 14.7%. The IGF-I samples were ran in duplicate and determinations were considered for re-runs if the coefficient of variation between duplicate determinations was greater than 10%.

The quantification of circulating NEFA concentration was determined using triplicate 5 µL determinations via colorimetric assay using a commercially available kit that involved acyl-CoA stynthetase, acyl-CoA oxidase, and perioxidase in 96 well microtiter plates (NEFA-HR; Wako Diagnostics, Richmond, VA). The NEFA assay was performed using sera from each individual steer (n = 2 steers/ pen) and these values were averaged together prior to statistical analysis. The standard curve constructed for the NEFA assay was between 0 and 1.0 mEq/L. For the NEFA analysis, the intra-assay CV was 5.9% and the inter-assay CV was 7.3%. Samples were considered for re-runs if the coefficient of variation among the absorbance values for triplicate determinations was greater than 5%.

The quantification of circulating serum urea-N (SUN) concentration was determined

on a microplate spectrophotometer in triplicate 5  $\mu$ L determinations, using diacetylmonoxime via a commercially available kit (STANBIO Urea Nitrogen-0580; STANBIO Laboratory, Boerne, TX). The SUN assay was performed using sera from each individual steer (n = 2 steers/pen) and these values were averaged together prior to statistical analysis. The standard curve constructed for the SUN assay was between 0 and 25.0 mg/dL. For the SUN analysis the intra-assay CV was 6.6% and the inter-assay CV was 10.4%. Samples were considered for re-runs if the coefficient of variation among the absorbance values for triplicate determinations was greater than 5%.

Circulating 17β-TbOH concentration was quantified via liquid chromatography-tandem mass spectrometry (LC-MS/MS) using slight modifications to the procedures described by (Blackwell et al., 2014). The 17β-TbOH assay was conducted using sera pooled by pen and day, whereas sera from all steers in NI were pooled by day, and not included in the statistical analysis for circulating 17β-TbOH concentrations. In 15 mL conical screw top tubes, equal volumes of methyl-tert-butyl-ether (MTBE) and sera (2 mL) were spiked with 10 ng of internal standard (17β-trenbolone-d3, National Institute for Public Health and the Environment of the Netherlands), then placed on an orbital shaker at 300 rpm for 30 min at room temperature. Samples were then centrifuged at room temperature for 5 min at  $1500 \times g$  in order to separate sera and MTBE layers. The MTBE layer was removed and then transferred to  $100 \times 16$  mm borosilicate glass tubes and evaporated to dryness at 35 °C under a gentle stream of nitrogen. Samples were reconstituted in 4 mL 80:20 methanol:water (HPLC grade, Fisher Scientific, Hampton, NH). Next, 3 mL of Hexane (HPLC grade, Fisher Scientific) was added to the reconstituted samples and samples were vortexed for 30 s. Following the vortex step, samples were centrifuged at room temperature for 5 min at  $1500 \times g$  in order to separate the water:methanol mixture from the hexane layer, the hexane layer (top) was then discarded, and the hexane wash was repeated. Samples were then dried to a volume of less than 0.5 mL under a gentle stream of nitrogen at 35 °C, and 3 mL of 5:95 methanol:water + 0.1% ammonium hydroxide was added to each sample prior to SPE cleanup. Oasis MAX cartridges (3cc/60 mg; Waters Corporation, Milford, MA) were conditioned with 3 mL of methanol and 3 mL of 5:95 methanol:water + 0.1% ammonium hydroxide, samples were passed through, and cartridges were washed with  $2 \times 3 \text{ mL}$ 5:95 methanol:water + 0.1% ammonium hydroxide.

Cartridges were then allowed to dry under vacuum for 10 min, and samples were eluted into clean  $16 \times 100$  mm borosilicate glass tubes with 7 mL of methanol. The samples were then evaporated to dryness at 35°C under a gentle stream of nitrogen and reconstituted in 100 µL of 60:40 methanol:water. The reconstituted sample was passed through a 0.45 µM polypropylene filter into fixed-insert microvials, capped, and stored at -20 °C until analysis. Blank (n = 3) and spiked (n = 3) matrix (bovine serum, Sigma-Aldrich, St. Louis, MO) samples were analyzed along with 42 unknowns per sample batch (48 extractions in total) in order to monitor extraction method performance. No steroids were observed above the limit of detection in any solvent or matrix blank. The mean matrix spike recovery (n = 27) for sera was  $(93 \pm 15.5 \%)$ 

Quantification of  $17\beta$ -TbOH was performed via triple quadrupole LC-MS/MS (TSQ Endura, ThermoFisher). Chromatography was performed using a methanol:water gradient elution taken from Blackwell et al. (2013) and a Gemini-NX C18 column (150 × 2.0 mm; Phenomenex, Torrance, CA) with a sample injection volume of 10 µL. Ionization was performed using atmospheric pressure chemical ionization in positive mode. Solvent blanks and check standards were included every 8 and 16 samples, respectively, in instrument runs for QC purposes. The limit of quantification, as determined by the lowest calibration standard included in sample runs, was 25 pg/mL serum.

## Removal

A total of 28 animals were removed during the course of the study. Two animals were removed from the NI treatment for: symptoms consistent with mycoplasma bovis (1) and for musculoskeletal issues not related to treatment (1). Seven animals were removed from the XR treatment due to: impalpable implants on d 35 (5), Hardware disease (1), and chronic infection at the site where the ear tag was applied (1). Nine animals were removed from the XS treatment due to: impalpable implants on d 35 (4) and d 70 (1), chronic Bovine Respiratory Disease Complex (2), symptoms consistent with mycoplasma bovis (1), and irresolvable footrot (1). Eight animals were removed from the E200 treatment due to: impalpable implants on d 35 (4) and d 70 (2), only steer left in his pen following removal of other steers (1), and broken lumbar vertebrae (1). Two animals were removed from the D200 treatment due to: apparent metabolic disorders (1) and musculoskeletal issues not related to treatment (1).

Cattle removed from the experiment due to impalpable implants were removed from their home pens on d 105. Their contribution to the pen mean body weight was deleted from the onset of the experiment, and total DMI for the pen was not adjusted to reflect this, as it was assumed that the removed steers had consumed the average DMI of the pen up until the point of their removal.

The body weight contributions to the pen mean for steers removed from the study for other health issues were removed back to the nearest interim weigh day, and feed intake from the previous weigh day up to the point of animal removal was subtracted from total feed delivered to that pen. It was weighted on a per-animal, per-day basis and applied to appropriate pens accordingly.

One XR pen was removed from the statistical analysis for sera metabolites because two of the removed steers in this individual pen were steers selected for blood collection, and one E200 pen was removed entirely from the statistical analysis for all variables since three removed animals came from that individual pen.

## **Carcass Evaluation**

When treatment blinded personnel estimated approximately 60% of the steers had external fat cover sufficient to grade USDA Choice, all cattle were transported 209 km to a commercial abattoir (Tyson Fresh Meats, Amarillo, TX). Trained personnel (West Texas A&M University) collected all carcass measurements. During the harvest process, any carcass trim, and fat and hide pulls of soft tissue  $\geq$ 6.8 kg, were noted. Individual carcass measurements (n = 212) included HCW, 12th-rib BF depth, LM area, KPH %, marbling score, and USDA quality grade. Yield grade was calculated by using the USDA regression equation (USDA, 1997). The percentage of EBF and final BW adjusted to 28% EBF (AFBW) were both generated using equations described by Guiroy et al. (2002). A 1.5% shrink was applied to d 213 BW for calculation of dressing percentage.

#### Statistical Analysis

Live performance and carcass data continuous in nature were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC), considering pen as the experimental unit. The statistical model included the fixed effect of implant treatment, while block was considered as a random effect. Least-squares means were generated using the LSMEANS statement of SAS. Data were separated and denoted to be different using the pairwise comparisons PDIFF and LINES option of SAS when a significant preliminary F-test was detected. All data categorical in nature were analyzed via chisquare analysis in SAS. An  $\alpha$  level of 0.05 was used to determine significance, with tendencies discussed at *P* values between 0.05 and 0.10.

Relative gain responses and sera metabolite data were analyzed as a randomized complete block design with repeated measures over time using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Pen was considered the experimental unit for sera metabolite data. The statistical model for the effect of implant treatment and day of study for sera metabolites included the fixed effect of implant, day, and the interaction of implant × day. Block was included as a random effect. A repeated statement was used and included day as the repeated variable. The covariance structure with the lowest Akaike information criterion (AIC) was used (Littell et al., 1998). All results are reported as least-squares means. Data were separated using the PDIFF option of SAS if a significant preliminary F-test was detected. In order to account for the influence of numerical differences across implant treatment for d 0 IGF-I concentrations, a covariate of d 0 IGF-I concentration was used in the model for analysis of sera IGF-I concentrations. An  $\alpha$ level of 0.05 was used to determine significance, with tendencies discussed at P values between 0.05 and 0.10.

#### RESULTS

#### Steer Performance

An implant × day interaction (P < 0.01) was detected for relative gain responses to the negative control treatment (Figure 1.). Values for relative gain response differed as a result of implant treatment on different days. On d 70 steers from XS and E200 had greater (P < 0.05) relative gain responses over NI, XR, and D200. From d 71 to 140 steers from XR, XS, and D200 had greater (P < 0.05) relative gain responses over NI, while steers in E200 were intermediate (P > 0.10) and did not differ from others. From d 141 to 213 no differences (P >0.10) were detected among treatments for relative gain responses.

Cumulative live performance data for steers can be found in (Table 2). No differences (P > 0.10) were detected for initial on test BW between treatments. Final live BW measures of all implanted



**Figure 1.** Effect of implant treatment on relative gain response over negative control (NI) in finishing steers (pooled standard error of the mean = 3.849; n = 12 pens/treatment, except for E200 where n = 11 pens/treatment). Treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg E<sub>2</sub> (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg E<sub>2</sub> (noncoated), 120 mg TBA + 24 mg E<sub>2</sub> (coated), 200 mg TBA + 40 mg E<sub>2</sub> (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg E<sub>2</sub> (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200).

steers were greater (P < 0.05) than NI steers. Final live BW was greatest for the XS treatment, and XS steers achieved a greater (P < 0.05) final live BW by 22 and 32 kg over XR and E200 steers, respectively. Final live BW for D200 steers was intermediate, and not different (P > 0.10) from XS, XR, or E200. Implanted steers achieved a greater cumulative live ADG (P < 0.05) than NI steers. The use of implants increased cumulative live ADG by 17% over NI steers. Cumulative live ADG was greatest for the XS treatment, and XS had increased (P < 0.05) ADG by 7.5 and 11% over XR and E200 steers, respectively, while cumulative live ADG for D200 steers was intermediate, and not different (P> 0.10) from XS, XR, or E200 treatment groups. Implants stimulated DMI in the present study, the XS treatment group had the greatest DMI throughout the course of the 213 d study and daily DMI

	NI	Imp	lant <sup>2</sup>		D200	
		XR	XS	E200		SEM
No. of steers	46	41	39	40	46	-
No. of pens	12	12	12	11	12	-
Initial BW, kg <sup>3</sup>	306	306	305	305	305	5.1
Live Basis						
Final BW, kg <sup>4</sup>	573°	612 <sup>b</sup>	634 <sup>a</sup>	602 <sup>b</sup>	619 <sup>ab</sup>	8.6
ADG, kg	1.25 <sup>c</sup>	1.43 <sup>b</sup>	1.54ª	1.39 <sup>b</sup>	1.47 <sup>ab</sup>	0.032
DMI, kg	8.16 <sup>c</sup>	8.57 <sup>b</sup>	9.16 <sup>a</sup>	8.46 <sup>bc</sup>	8.77 <sup>ab</sup>	0.169
G:F	0.153 <sup>b</sup>	$0.167^{a}$	0.168ª	0.164 <sup>a</sup>	0.168ª	0.0030
Carcass Adjusted <sup>5</sup>						
Final BW, kg	585 <sup>d</sup>	625 <sup>bc</sup>	662ª	608°	633 <sup>b</sup>	10.3
ADG, kg	1.31 <sup>d</sup>	1.50 <sup>bc</sup>	1.67ª	1.42°	1.54 <sup>b</sup>	0.081
G:F	0.160 <sup>c</sup>	$0.175^{ab}$	0.183ª	0.168 <sup>bc</sup>	0.175 <sup>ab</sup>	0.0035

Table 2. Cumulative performance on live and carcass weight adjusted basis<sup>1</sup>

<sup>1</sup>Least squares means.

<sup>2</sup>Implant treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg  $E_2$  (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg  $E_2$  (noncoated), 120 mg TBA + 24 mg  $E_2$  (coated), 200 mg TBA + 40 mg  $E_2$  (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**XS**), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**E200**), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 (**D200**).

<sup>3</sup>Initial BW with 1.5% shrink.

<sup>4</sup>Final BW (d213) with 1.5% shrink.

<sup>5</sup>HCW/0.625.

<sup>a,b</sup>Means within a row without a common superscript differ (P < 0.05).

for XS was greater (P < 0.05) than NI, XR, or E200 treatment groups. However, daily DMI for the XS steers was not different (P > 0.10) from D200 steers (9.16 vs.  $8.77 \pm 0.169$  kg), for XS and D200, respectively. Daily DMI for D200 steers was numerically greater than, but not different (P >0.10) from the XR group (8.77 vs.  $8.57 \pm 0.169$  kg, for D200 and XR steers, respectively). The E200 steers consumed less (P < 0.05) DMI than D200 steers (8.46 vs.  $8.77 \pm 0.169$  kg, for E200 and D200 steers, respectively). Daily DMI for E200 steers was not different (P > 0.10) from XR or NI steers  $(8.46 \text{ vs. } 8.57 \text{ or } 8.16 \pm 0.169 \text{ kg}, \text{ for E200 vs. XR}$ and NI steers, respectively). The use of anabolic implants improved (P < 0.05) gain efficiency by 9% over NI steers (0.153 vs. 0.167, 0.168, 0.164, and  $0.168 \pm 0.0030$ ) throughout the 213 d study for NI, XR, XS, E200, and D200 steers, respectively.

Carcass-adjusted performance data can be found in (Table 2). Carcass-adjusted BW measures of all implanted steers were greater (P < 0.05) than NI steers. Carcass-adjusted BW was greatest for the XS treatment, and XS steers achieved a greater (P < 0.05) carcass-adjusted final BW by 37, 54, and 29 kg over XR, E200, and D200 steers, respectively. Carcass-adjusted final BW for E200 steers was less (P < 0.05) than D200 steers, and XR steers were intermediate and did not differ (P > 0.10) from E200 or D200 steers. Implanted steers achieved a greater carcass-adjusted ADG (P < 0.05) than NI steers. The use of implants increased carcass-adjusted ADG by 17% over NI steers. Carcassadjusted ADG was greatest for the XS treatment, and XS steers generated a greater (P < 0.05) carcass-adjusted ADG over XR, E200 and D200 steers. Although carcass-adjusted ADG for D200 steers was lower (P < 0.05) than XS steers, it was not different (P > 0.10) from XR steers, and carcass-adjusted ADG for D200 was increased (P < 0.05) by 8% over E200 steers. Carcass-adjusted ADG did not differ (P > 0.10) between XR and E200 treatment groups. Carcass-adjusted G:F was impacted by implant treatment, the NI steers had the greatest carcass-adjusted G:F, and carcass-adjusted G:F for NI was not different (P > 0.10) from E200 steers (0.160 vs. 0.168  $\pm$  0.0035). The E200 steers did not differ (P > 0.10) from XR or D200 steers for carcass-adjusted G:F; however, XS steers had improved carcass-adjusted G:F by 9% (P < 0.05) over E200, carcass-adjusted G:F for XS versus XR or D200 did not differ (P > 0.10).

Interim performance data for steers can be found in (Table 3). During the initial 70 d period following study initiation, the XS and E200 steers had greater (P < 0.05) ADG over NI, XR, and D200 steers. Gain efficiency and GED was also improved  $(P \le 0.05)$  during the initial 70 d for XS and E200 steers over NI, XR, or D200 steers. From 71 to 140 d on test, the D200 steers achieved a greater (P < 0.05) ADG by 50, 7, and 26% over NI, XS, and E200 steers, respectively, while XR steers were intermediate and not different (P > 0.10) from XS, but were greater (P < 0.05) than E200 or NI steers. Gain efficiency was also improved (P < 0.05) from 71 to 140 d for XR and D200 steers over NI, XS, and E200 steers. Gain efficiency for XS steers was intermediate and improved (P < 0.05) by 21 and 8% from NI and E200 steers, respectively. The NI steers had the poorest G:F from 71 to 140 d and G:F was greater (P < 0.05) for E200 than NI steers. Gain energy density of live weight gain was also lower (P < 0.05) from 71 to 140 d for XR and D200 steers over NI, XS, and E200 steers. The GED values for XS and E200 steers were intermediate and both differed from ( $P \le 0.05$ ) NI steers. From 141 to 213 d on test, the XR steers achieved a greater (P < 0.05) ADG by 14 and 22% over NI and E200 steers, respectively, whereas XS and D200 steers were intermediate and not different (P > 0.10) from XR or NI, but both were greater (P < 0.05) than E200 steers. Gain efficiency was improved (P < 0.05) from 141 to 213 d for XR and NI steers over E200 steers. Gain efficiency from 141 to 213 d for XS and D200 steers were intermediate and did not differ from (P > 0.10) NI or E200 steers. Gain energy density of live weight gain was also improved (P < 0.05) by 20% from 141 to 213 d for XR over XS steers. The GED values from 141 to 213 d for NI, E200, and D200 steers were intermediate and did not differ (P > 0.10) from XR or XS steers.

#### **Carcass Data**

Carcass data for these steers can be found in (Table 4). The use of implants increased HCW by 8% compared to NI steers. Hot carcass weight measures for all implanted steers were greater (P < 0.05) than NI steers. Steers in the XS group had greater (P < 0.05) HCW by 23, 34, and 18 kg over XR, E200, and D200 steers, respectively. The D200 steers had similar HCW (P > 0.10) to XR steers (396 vs. 391 ± 6.4 kg for D200 and XR, respectively), and D200 achieved a 16-kg greater (P < 0.05) HCW over E200 steers, whereas XR did not differ (P > 0.10) from D200 or E200 steers. The XS steers had the greatest (P < 0.05) dressing percentage. Overall dressing percentage between NI, XR, E200, and D200 groups did not differ (P > 0.10). Steers in the

Table 3. Interir	n steer performance	responses <sup>1,2</sup>
------------------	---------------------	--------------------------

		Imp	olant <sup>3</sup>			
	NI	XR	XS	E200	D200	SEM
No. of steers	46	41	39	40	46	-
No. of pens	12	12	12	11	12	-
Initial BW, kg	311	311	310	310	310	5.1
Initial to 70d						
d 70 BW, kg	424 <sup>b</sup>	419 <sup>ь</sup>	443ª	440 <sup>a</sup>	420 <sup>b</sup>	6.6
ADG, kg	1.61 <sup>b</sup>	1.50 <sup>b</sup>	1.90ª	1.85 <sup>a</sup>	1.57 <sup>b</sup>	0.050
DMI, kg	8.29 <sup>ab</sup>	8.13 <sup>b</sup>	8.58ª	8.16 <sup>b</sup>	8.27 <sup>ab</sup>	0.171
G:F	0.194 <sup>b</sup>	0.185 <sup>b</sup>	0.221ª	0.227ª	0.190 <sup>b</sup>	0.0052
GED, Mcal/kg	5.12 <sup>a</sup>	5.26ª	4.38 <sup>b</sup>	4.22 <sup>b</sup>	5.12 <sup>a</sup>	0.155
71 to 140d						
d 140 BW, kg	510°	540 <sup>ь</sup>	565ª	544 <sup>b</sup>	551 <sup>ab</sup>	7.9
ADG, kg	1.24 <sup>d</sup>	1.77 <sup>ab</sup>	1.75 <sup>b</sup>	1.49°	1.87ª	0.043
DMI, kg	8.49°	8.84 <sup>bc</sup>	9.63ª	9.02 <sup>b</sup>	8.92 <sup>b</sup>	0.191
G:F	0.146 <sup>d</sup>	0.200ª	0.181 <sup>b</sup>	0.165°	0.210 <sup>a</sup>	0.0037
GED, Mcal/kg	6.04 <sup>a</sup>	4.44°	5.01 <sup>b</sup>	5.41 <sup>b</sup>	4.19°	0.150
141 to 213d						
d 213 BW, kg	582°	621 <sup>b</sup>	644ª	611 <sup>b</sup>	629 <sup>ab</sup>	8.7
ADG, kg	0.98 <sup>bc</sup>	1.12 <sup>a</sup>	$1.08^{ab}$	0.92°	$1.07^{ab}$	0.049
DMI, kg	7.73°	8.73 <sup>ab</sup>	9.25ª	8.24 <sup>bc</sup>	9.11 <sup>a</sup>	0.215
G:F	0.127 <sup>ab</sup>	0.128ª	0.117 <sup>bc</sup>	0.111°	0.117 <sup>bc</sup>	0.0049
GED, Mcal/kg	6.41 <sup>ab</sup>	6.17 <sup>b</sup>	7.42ª	6.96 <sup>ab</sup>	7.17 <sup>ab</sup>	0.442

<sup>1</sup>Nonshrunk BW basis.

<sup>2</sup>Least squares means.

<sup>3</sup>Implant treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg  $E_2$  (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg  $E_2$  (noncoated), 120 mg TBA + 24 mg  $E_2$  (coated), 200 mg TBA + 40 mg  $E_2$  (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**XS**), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**E200**), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 (**D200**).

<sup>a,b</sup>Means within a row without a common superscript differ (P < 0.05).

XS group had greater (P < 0.05) BF over NI, XR, and D200 steers. The E200 steers had similar (P >0.10) BF to XS steers, and BF measures for E200 steers did not differ (P > 0.10) from NI, XR, or D200 steers. Ribeye area did not differ (P > 0.10) across treatments; however, XR had an increase of 5% in LM area over NI and E200 steers. The NI steers had greater (P < 0.05) marbling scores over E200 and D200 steers, marbling scores for XR and XS steers were intermediate and did not differ (P >0.10) from NI, E200, or D200 steers. Kidney, pelvic, and heart fat % was the greatest for E200 steers, and KPH values for E200 were greater (P < 0.05) than NI steers. Steers from XR, XS, and D200 were intermediate for KPH and did not differ (P > 0.10)from NI or E200. The XS steers had the greatest (P < 0.05) calculated yield grade over all other treatments. Calculated yield grade between NI, XR, E200, and D200 groups did not differ (P > 0.10). The XS steers had the greatest calculated EBF and calculated EBF for XS was increased (P < 0.05) by 8.3, 6.1, 6.1, and 5.7% from NI, XR, E200, and D200, respectively. The XR, XS, and D200 steers had greater (P < 0.05) AFBW over NI steers, and E200 steers were intermediate and did not differ (P > 0.10) from NI, XR, XS, or D200. There were no differences (P > 0.10) detected among treatments for yield or quality grade distribution.

#### Sera Metabolite Data

An implant × day interaction was detected (P < 0.01) for circulating  $E_2$  concentrations (Table 5; Figure 2). Sera  $E_2$  concentrations were not different (P > 0.10) among treatments at study initiation. At 14 d, poststudy initiation steers in the E200 group had elevated (P < 0.05)  $E_2$  over all other treatments. The  $E_2$  values between NI, XR, XS, and D200 steers did not differ (P > 0.10) on d 14. At 35 d, poststudy initiation steers in the E200 group had elevated (P < 0.05)  $E_2$  concentrations over all other treatments. The XS steers had greater (P < 0.05) circulating  $E_2$  concentrations over NI, XR, and D200 steers on d 35. Circulating  $E_2$  concentrations for NI, XR, and D200 did not differ (P > 0.10) on d 35. At 70 d, poststudy initiation steers

		Imp	lant <sup>2</sup>			
	NI	XR	XS	E200	D200	SEM
Final BW, kg <sup>3</sup>	573°	612ь	634 <sup>a</sup>	602ь	619 <sup>ab</sup>	8.6
HCW, kg	366 <sup>d</sup>	391 <sup>bc</sup>	414 <sup>a</sup>	380°	396 <sup>b</sup>	6.4
Dress, %	63.77 <sup>b</sup>	63.86 <sup>b</sup>	65.22ª	63.08 <sup>b</sup>	63.87 <sup>b</sup>	0.446
Back fat, cm	1.22 <sup>b</sup>	1.35 <sup>b</sup>	1.63ª	1.42 <sup>ab</sup>	1.37 <sup>b</sup>	0.074
LM area, cm <sup>2</sup>	90.19	94.84	91.23	90.32	92.39	1.981
Marbling	492ª	480 <sup>ab</sup>	464 <sup>ab</sup>	438 <sup>b</sup>	445 <sup>b</sup>	15.2
КРН, %	2.18 <sup>b</sup>	2.26 <sup>ab</sup>	2.25 <sup>ab</sup>	2.28ª	2.24 <sup>ab</sup>	0.035
YG	2.74 <sup>b</sup>	2.83 <sup>b</sup>	3.48 <sup>a</sup>	3.05 <sup>b</sup>	3.03 <sup>b</sup>	0.130
EBF, % <sup>4</sup>	29.04 <sup>b</sup>	29.64 <sup>b</sup>	31.44 <sup>a</sup>	29.63 <sup>b</sup>	29.74 <sup>b</sup>	0.429
AFBW, kg <sup>5</sup>	559 <sup>b</sup>	587ª	592ª	572 <sup>ab</sup>	592ª	8.9
Y2, %	75.00	66.70	16.70	36.40	50.00	
Y3, %	25.00	33.30	75.00	63.60	41.70	Chi Sq. <i>P</i> = 0.13
Y4, %	0.00	0.00	8.30	0.00	8.30	
Select, %	0.00	8.30	8.30	27.30	16.70	
Low Choice, %	50.00	41.70	66.70	63.60	66.60	Chi Sq. <i>P</i> = 0.22
Choice Plus, %	50.00	50.00	25.00	9.10	16.70	

#### Table 4. Carcass trait responses<sup>1</sup>

<sup>1</sup>Least squares means.

<sup>2</sup>Implant treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg  $E_2$  (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg  $E_2$  (noncoated), 120 mg TBA + 24 mg  $E_2$  (coated), 200 mg TBA + 40 mg  $E_2$  (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**XS**), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**E200**), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 (**D200**).

<sup>3</sup>1.5% pencil shrink applied to Final BW.

<sup>4</sup>Empty Body Fat% according to equations described by Guiroy et al. (2002; Journal of Animal Science 80: 1791).

<sup>5</sup>Final BW at 28 % EBF, Guiroy et al. (2002; Journal of Animal Science 80: 1791).

<sup>a,b</sup>Means within a row without a common superscript differ (P < 0.05).

in the E200 and XS had increased (P < 0.05)  $E_2$  concentrations over NI and D200 steers; whereas circulating  $E_2$  values for steers in XR were intermediate, not differing (P > 0.10) from NI, XS, E200, or D200. Circulating  $E_2$  was greatest (P < 0.05) for D200 over all other treatments on d 105 (22.27 vs.

0.91, 9.49, 9.97, 5.74  $\pm$  1.063 pg/mL) for NI, XR, XS, and E200, respectively; the NI steers had lower (P < 0.05) circulating E<sub>2</sub> concentrations relative to XR, XS, and E200. The XS steers had greater (P < 0.05) circulating E<sub>2</sub> concentrations than E200 steers (9.97 vs. 5.74  $\pm$  1.063 pg/mL), while XR was

Table 5. Effects of implant (IM)	on sera estradiol-17 $\beta$ (pg/mL)
----------------------------------	--------------------------------------

			Implant (IM)	1		P	alue		
	NI	XR	XS	E200	D200	SEM <sup>2</sup>	IM	D	IM × d
Days rel	lative to study i	nitiation				1.063	< 0.01	< 0.01	< 0.01
0	4.43	2.83	3.72	3.66	2.26				
14	3.03 <sup>b</sup>	2.01 <sup>b</sup>	7.77 <sup>b</sup>	11.39 <sup>a</sup>	2.85 <sup>b</sup>				
35	3.34°	1.50°	5.76 <sup>b</sup>	$10.97^{a}$	1.69°				
70	1.73 <sup>b</sup>	4.99 <sup>ab</sup>	7.68 <sup>a</sup>	9.01 <sup>a</sup>	0.92 <sup>b</sup>				
105	0.91 <sup>d</sup>	9.49 <sup>bc</sup>	9.97 <sup>b</sup>	5.74°	27.27 <sup>a</sup>				
140	1.07°	6.38 <sup>b</sup>	5.59 <sup>b</sup>	2.43 <sup>bc</sup>	22.98 <sup>a</sup>				
175	0.62°	3.69 <sup>b</sup>	3.35 <sup>b</sup>	1.15°	8.43 <sup>a</sup>				
213	1.26 <sup>c</sup>	2.43 <sup>bc</sup>	4.12 <sup>b</sup>	1.24 <sup>c</sup>	6.18 <sup>a</sup>				

<sup>1</sup>Implant treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg  $E_2$  (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg  $E_2$  (noncoated), 120 mg TBA + 24 mg  $E_2$  (coated), 200 mg TBA + 40 mg  $E_2$  (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**E200**), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 (**D200**).

<sup>2</sup>Pooled standard error of implant by day treatment means; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment).

<sup>a,b</sup>Means within a row without a common superscript differ (P < 0.05).



**Figure 2.** Effect of implant treatment on sera estradiol-17 $\beta$  (E<sub>2</sub>) concentrations in finishing steers (pooled standard error of the mean = 1.063; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment). Treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg E<sub>2</sub> (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg E<sub>2</sub> (noncoated), 120 mg TBA + 24 mg E<sub>2</sub> (coated), 200 mg TBA + 40 mg E<sub>2</sub> (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg E<sub>2</sub> (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200).

intermediate, not differing (P > 0.10) from XS or E200 on d 105. Steers from the D200 group had the greatest (P < 0.05) circulating E<sub>2</sub> levels on d 140 relative to steers in NI, XR, XS, or E200. Steers from XR and XS had elevated (P < 0.05) circulating E<sub>2</sub> concentrations over NI on d 140, whereas E200 steers were intermediate, not differing (P > 0.10) from NI, XR, or XS. Steers in D200 had the greatest (P < 0.05) circulating E<sub>2</sub> levels over all other treatments on d 175. Steers in XR and XS were similar and did not differ (P > 0.10) on d 175 (3.69 vs.  $3.35 \pm 1.063$  pg/mL for XR and XS, respectively), and both were greater (P < 0.05) than NI and E200 steers that were both similar (P > 0.10) on d 175 (0.62 vs. 1.15 ± 1.063 pg/mL for NI and E200, respectively). The D200 steers had the greatest (P < 0.05) concentrations of circulating E<sub>2</sub> over all other treatments on d 213. On d 213, steers in XS had greater (P < 0.05) E<sub>2</sub> concentrations in sera compared to steers from NI and E200, whereas steers in XR were intermediate (P > 0.10)not differing from NI, XS, or E200.

Using d 0 as a covariate (P < 0.01), an implant × day interaction was detected (P < 0.01) for sera IGF-I concentrations (Table 6; Figure 3). Sera IGF-I concentrations were not different (P > 0.10) among implant groups on d 14. At 35 d post study initiation, steers in the XS group had increased (P < 0.05) sera IGF-I concentrations over NI, XR, and D200 steers, whereas E200 steers were intermediate and did not differ (P > 0.10) from NI, XR, D200, or XS. Steers in the XS group had increased (P < 0.05) circulating IGF-I values over NI, XR and D200 steers on d 70. Sera IGF-I levels for the E200 steers was greater than (P < 0.05)

NI and D200, whereas XR steers were intermediate and did not differ (P > 0.10) from D200 or NI steers. At d 105, all implanted groups had greater (P < 0.05) circulating IGF-I concentrations over NI steers. On d 105, the D200 steers had greater (P < 0.05) sera IGF-I levels over XS steers (472.6 vs.  $388.6 \pm 27.42$  ng/mL), whereas XR and E200 were intermediate and did not differ (P > 0.10) from D200 or XS. Circulating IGF-I concentrations on d 140 were greater (P < 0.05) for XR, E200, and D200 than NI; whereas XS was intermediate and did not differ (P > 0.10) from NI, XR, E200, or D200 steers. At d 175, all implanted steers had greater (P < 0.05) sera IGF-I levels over NI steers. Steers in the D200 group had increased (P < 0.05) circulating IGF-I concentrations over XS steers  $(360.0 \text{ vs. } 288.0 \pm 27.42 \text{ ng/mL})$  on d 175; the XR and E200 steers were intermediate and not different (P > 0.10) from D200 or XS steers. On d 213, all implanted steers had elevated (P < 0.05) sera IGF-I levels relative to NI steers.

No implant × day interaction was noted (P > 0.10) for circulating NEFA concentrations (Table 7; Figure 4). Sera NEFA concentrations did not differ (P > 0.10) as a result of implant treatment; however, sera NEFA concentrations differed over time (P < 0.01).

An implant × day interaction was noted (P < 0.01) for circulating concentrations of SUN (Table 8; Figure 5). Serum urea-N concentrations were not different (P > 0.10) among treatments at study initiation. At 14 d, poststudy initiation steers in the E200 and XS groups had decreased (P < 0.05) SUN levels over D200 steers, whereas NI and XR steers did not differ (P > 0.10) from XS steers,

			Implant (IM) <sup>1</sup>		P	alue			
	NI	XR	XS	E200	D200	SEM <sup>2</sup>	IM	d	IM × d
Days re	lative to study in	nitiation				28.01	< 0.01	< 0.01	< 0.01
IGF-I v	vith day 0 as a c	ovariate <sup>3</sup>							
14	317.4	315.0	361.6	361.7	301.2				
35	311.1 <sup>b</sup>	318.6 <sup>b</sup>	413.4 <sup>a</sup>	379.4 <sup>ab</sup>	315.5 <sup>b</sup>				
70	271.0 <sup>c</sup>	366.7 <sup>bc</sup>	457.3ª	447.6 <sup>ab</sup>	341.3°				
105	288.8°	443.3 <sup>ab</sup>	383.0 <sup>b</sup>	441.6 <sup>ab</sup>	467.7 <sup>a</sup>				
140	292.2°	402.0 <sup>ab</sup>	349.9 <sup>bc</sup>	433.8 <sup>a</sup>	415.3 <sup>ab</sup>				
175	206.9°	313.7 <sup>ab</sup>	282.4 <sup>b</sup>	297.0 <sup>ab</sup>	355.0 <sup>a</sup>				
213	206.8 <sup>b</sup>	328.5ª	293.6 <sup>a</sup>	291.6 <sup>a</sup>	301.2 <sup>a</sup>				

Table 6. Effects of implant (IM) on sera IGF-I (ng/mL)

<sup>1</sup>Implant treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg  $E_2$  (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg  $E_2$  (noncoated), 120 mg TBA + 24 mg  $E_2$  (coated), 200 mg TBA + 40 mg  $E_2$  (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**XS**), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**E200**), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 (**D200**).

<sup>2</sup>Pooled standard error of implant by day treatment means; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment). <sup>3</sup>Day 0 IGF-I as a covariate (P < 0.01).

<sup>a,b</sup>Means within a row without a common superscript differ (P < 0.05).

they had elevated (P < 0.05) SUN values relative to E200 steers. The SUN values between NI, XR, and D200 steers did not differ (P > 0.10) on d 14. At 35 d, posttreatment initiation steers in the E200 group had decreased (P < 0.05) SUN values over all other treatments. At 70 d, posttreatment initiation steers in the E200 and XR groups had decreased (P < 0.05) SUN over NI steers, whereas XS and D200 steers were intermediate and did not differ (P > 0.10) from E200, XR, or NI steers. Serum urea-N values among treatments were similar (P > 0.10) on d 105, 140, and 175. On d 213 there tended to a difference (P < 0.10) among implant treatments for SUN values, in which E200 steers had lower SUN compared to steers in the XR group, while NI, XS, and D200 steers were intermediate and did not differ (P > 0.10) from E200 or XR steers.

An implant × day interaction was detected (P < 0.01) for circulating 17 $\beta$ -TbOH concentrations (Table 9; Figure 6). Sera 17 $\beta$ -TbOH concentrations were not different (P > 0.10) among treatments at treatment initiation. By 14 d, post-treatment initiation steers in the E200 and XS groups had the greatest (P < 0.05) sera 17 $\beta$ -TbOH concentrations over all other treatments. At 35 d, posttreatment initiation steers in the E200 group had elevated (P < 0.05) circulating 17 $\beta$ -TbOH concentrations over all other treatments. At 70



Figure 3. Effect of implant treatment on sera IGF-I concentrations in finishing steers using day 0 IGF-I concentrations as a covariate (P < 0.01; pooled standard error of the mean = 28.01; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment). Treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg E<sub>2</sub> (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg E<sub>2</sub> (noncoated), 120 mg TBA + 24 mg E<sub>2</sub> (coated), 200 mg TBA + 40 mg E<sub>2</sub> (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg E<sub>2</sub> (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200).

Table 7. Effects of impla	nt (IM) on sera	NEFA (mEq/L)
---------------------------	-----------------	--------------

		Implant (IM) <sup>1</sup>					<i>P</i> value			
	NI	XR	XS	E200	D200	SEM <sup>2</sup>	IM	d	IM × d	
Days re	lative to study in	itiation				0.04363	0.86	< 0.01	0.96	
0	0.4088	0.3803	0.4358	0.3905	0.3992					
14	0.2884 <sup>b</sup>	0.2681 <sup>b</sup>	0.2733 <sup>b</sup>	0.3645 <sup>a</sup>	0.2838 <sup>b</sup>					
35	0.2646	0.2585	0.2873	0.3000	0.2548					
70	0.3802	0.3206	0.3422	0.3157	0.3037					
105	0.2769	0.3059	0.3075	0.3305	0.3143					
140	0.3354	0.3087	0.3201	0.3224	0.3323					
175	0.2800	0.2708	0.2419	0.2712	0.2735					
213	0.3754	0.4003	0.3669	0.3736	0.3709					

<sup>1</sup>Implant treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg  $E_2$  (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg  $E_2$  (noncoated), 120 mg TBA + 24 mg  $E_2$  (coated), 200 mg TBA + 40 mg  $E_2$  (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**XS**), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**E200**), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 (**D200**).

<sup>2</sup>Pooled standard error of implant by day treatment means; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment). <sup>a,b</sup>Means within a row without a common superscript differ (P < 0.05).

d, posttreatment initiation steers in XR, XS, and E200 had increased (P < 0.05) circulating 17 $\beta$ -TbOH levels over D200 steers. On d 105, 140, 175, and 213, the D200 steers had the greatest (P < 0.05) circulating 17 $\beta$ -TbOH concentrations over all other treatments.

# DISCUSSION

# **Performance Responses to Implant Treatment**

The primary objective of this experiment was to compare coated implants administered 213 d prior to harvest to conventional noncoated implants administered 213 or 143 d prior to harvest in beef steers. The use of implants, regardless of coating, total dose, or timing of administration increased final live BW measures relative to NI steers. The use of XS in the present study provided equal doses of anabolic hormone associated with an initial implant and re-implant protocol: initial Revalor-IS [80 mg TBA + 16 mg  $E_2$  (Merck Animal Health)] and re-implanted with Revalor-S [120 mg TBA + 24 mg  $E_2$  (Merck Animal Health)] approximately 75 d later. The Revalor-XS implant has been thoroughly compared to single and re-implant protocols previously (Parr et al., 2011a; Parr et al., 2011b; and Nichols et al., 2014). The altered payout characteristics associated with XS improved final live BW measures over XR and E200 which is consistent



**Figure 4.** Effect of implant treatment on sera NEFA concentrations in finishing steers (pooled standard error of the mean = 0.04363; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment). Treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg E<sub>2</sub> (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg E<sub>2</sub> (noncoated), 120 mg TBA + 24 mg E<sub>2</sub> (coated), 200 mg TBA + 40 mg E<sub>2</sub> (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg E<sub>2</sub> (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200).

		Implant (IM) <sup>1</sup>					Р	value	
	NI	XR	XS	E200	D200	SEM <sup>2</sup>	IM	d	IM × d
Days rel	ative to study in	itiation				0.670	0.01	< 0.01	0.01
0	9.46	9.04	9.46	8.82	8.50				
14	8.18 <sup>ab</sup>	8.14 <sup>ab</sup>	7.30 <sup>bc</sup>	5.81°	9.45ª				
35	$8.00^{a}$	$8.70^{a}$	8.43ª	5.93 <sup>b</sup>	8.75 <sup>a</sup>				
70	10.03 <sup>a</sup>	7.13 <sup>b</sup>	8.75 <sup>ab</sup>	6.95 <sup>b</sup>	8.91 <sup>ab</sup>				
105	11.46	9.80	10.32	11.80	10.98				
140	11.85	11.33	12.49	11.71	11.51				
175	9.85	9.38	9.22	9.51	10.20				
213	9.12 <sup>gh</sup>	11.04 <sup>g</sup>	$9.78^{\mathrm{gh}}$	8.37 <sup>h</sup>	9.72 <sup>gh</sup>				

**Table 8.** Effects of implant (IM) on serum urea-N (mg/dL)

<sup>1</sup>Implant treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg  $E_2$  (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg  $E_2$  (noncoated), 120 mg TBA + 24 mg  $E_2$  (coated), 200 mg TBA + 40 mg  $E_2$  (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**E200**), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 (**D200**).

<sup>2</sup>Pooled standard error of implant by day treatment means; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment).

<sup>a,b</sup>Means within a row without a common superscript differ (P < 0.05).

<sup>g,h</sup>Means within a row without a common superscript differ (P < 0.10).

with others (Parr et al., 2011a, 2011b), but did not increase final live BW over steers receiving a noncoated TBA +  $E_2$  implant on d 70 (D200). In the present study, implants stimulated DMI and steers in the XS treatment group consumed the greatest daily DMI throughout the study. Additionally, the use of implants improved gain efficiency measures relative to NI steers. There were no differences for live basis gain efficiency detected in the present study in relation to the administration of one or the equivalent of two combination anabolic implants during the finishing phase, which is inconsistent with Reinhardt (2007). Furthermore, the use of XS in the present study increased carcass-adjusted BW measures over all other treatments, which is consistent with others (Parr et al., 2011a and 2011b). The reason that steers receiving equal amounts and ratios of TBA and  $E_2$  had differing carcass-adjusted ADG in the present study are likely associated with differing payout characteristics, due to differences in body weight, that resulted in differing absolute values of anabolic hormones in circulation. Steers in the E200 group had lower carcass-adjusted ADG values compared to D200 steers, whereas XR steers were intermediate and did not differ from E200 or D200. Reasons for differing responses could be explained by the fact that steers in the D200 group received anabolic stimulation at a time when DMI



**Figure 5.** Effect of implant treatment on serum urea-N concentrations in finishing steers (pooled standard error of the mean = 0.670; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment). Treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg E<sub>2</sub> (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg E<sub>2</sub> (noncoated), 120 mg TBA + 24 mg E<sub>2</sub> (coated), 200 mg TBA + 40 mg E<sub>2</sub> (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg E<sub>2</sub> (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200).

**Table 9.** Effects of implant (IM) on sera trenbolone- $17\beta$  (pg/mL)

			Implant (IM)		P value				
	NI <sup>2</sup>	XR	XS	E200	D200	SEM <sup>3</sup>	IM	d	IM × d
Days rel	ative to study	initiation				38.74	< 0.01	< 0.01	< 0.01
0	12.5	12.5	12.5	12.5	12.5				
14	12.5	12.5 <sup>b</sup>	215.7 <sup>a</sup>	269.2 <sup>a</sup>	12.5 <sup>b</sup>				
35	12.5	30.6 <sup>b</sup>	48.2 <sup>b</sup>	200.9 <sup>a</sup>	12.5 <sup>b</sup>				
70	12.5	138.8 <sup>a</sup>	135.2ª	138.0 <sup>a</sup>	12.5 <sup>b</sup>				
105	12.5	202.1 <sup>b</sup>	138.1 <sup>b</sup>	107.7 <sup>b</sup>	556.3ª				
140	12.5	118.2 <sup>b</sup>	85.7 <sup>b</sup>	34.4 <sup>b</sup>	408.5 <sup>a</sup>				
175	12.5	86.4 <sup>b</sup>	49.9 <sup>b</sup>	12.5 <sup>b</sup>	212.9 <sup>a</sup>				
213	12.5	29.1 <sup>b</sup>	29.6 <sup>b</sup>	21.8 <sup>b</sup>	96.2ª				

<sup>1</sup>Implant treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg  $E_2$  (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg  $E_2$  (noncoated), 120 mg TBA + 24 mg  $E_2$  (coated), 200 mg TBA + 40 mg  $E_2$  (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg  $E_2$  (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (**E200**), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 70 (**D200**).

 $^{2}$ Not included in statistical analysis, no 17 $\beta$ -trenbolone was detected in Negative controls pooled by day (sera from 12 pens pooled by day, 8 samples in total); since no 17 $\beta$ -trenbolone was detected values listed are one half the lower limit of detection of the assay.

<sup>3</sup>Pooled standard error of implant by day treatment means; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment).

<sup>a,b</sup>Means within a row without a common superscript differ (P < 0.05).

was maximized, while steers in the E200 group received maximal anabolic stimulation at a time when DMI had not reached the steers acclimated plateau. Also, increased BW results in increased intake needed for maintenance requirements, which coupled with decreased hormone payout late in the feeding period, might decrease cattle performance. Carcass-adjusted gain efficiency was the lowest for NI steers compared to XR, D200, and XS steers, which is consistent with (Parr et al., 2011a, 2011b). In the present study, the administration of a noncoated implant 213 d prior to harvest (i.e. E200) did not statistically improve carcass-adjusted G:F relative to NI. Furthermore, XS improved carcass-adjusted G:F over steers given a noncoated implant 213 d prior to harvest (i.e. E200), which is consistent with others (Parr et al., 2011a, 2011b), but did not improve carcass-adjusted G:F compared to XR or D200.

Interim performance was tabulated and analyzed in order to provide context to implant payout characteristics and the influences on gain, intake, and caloric density of live weight gain (GED). Additionally, relative gain response (Figure 1) relative to NI was tabulated by interim period, in order to demonstrate effective payout of the test



**Figure 6.** Effect of implant treatment on sera trenbolone-17 $\beta$  in finishing steers (pooled standard error of the mean = 38.74; Negative control pens were not included in statistical analysis; n = 12 pens/treatment, except for XR and E200 where n = 11 pens/treatment). Treatments were: Negative control given no implant (NI), Revalor-XR [200 mg TBA + 20 mg E<sub>2</sub> (coated), Merck Animal Health, Summit, NJ], administered subcutaneously in the center one-third of the ear on d 0 (XR), Revalor-XS [80 mg TBA + 16 mg E<sub>2</sub> (noncoated), 120 mg TBA + 24 mg E<sub>2</sub> (coated), 200 mg TBA + 40 mg E<sub>2</sub> (total), Merck Animal Health], administered subcutaneously in the center one-third of the ear on d 0 (XS), Revalor-200 [200 mg TBA + 20 mg E<sub>2</sub> (noncoated), Merck Animal Health] administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200), and Revalor-200 administered subcutaneously in the center one-third of the ear on d 0 (E200).

implants. During the initial 70 d on test, live BW measures and ADG were greatest for XS and E200 steers. Steers in the NI, XR, and D200 treatment groups were not different from one another for ADG during the initial 70 d of the feeding period. No difference were detected for daily gain or gain efficiency between XR and NI during the initial 70 d on test, and is similar to what has been reported previously by Merck Animal Health (FOIA, 2017). McLaughlin et al., (2013) reported that steers receiving a long-acting implant (coated implant: 200 mg TBA + 28 mg estradiol benzoate) had lower ADG and G:F when compared to steers given a conventional implant (noncoated implant: 200 mg TBA + 28 mg estradiol benzoate) during the initial 75 d of the study. Although steers administered a Revalor-XS were intermediate and did not differ from either implant group (McLaughlin et al., 2013), gain efficiency and GED measures were the lowest for XS and E200 steers during the initial 70 d on test in the present study. Steers in the NI, XR, and D200 treatment groups were not different from one another for gain efficiency or GED during the initial 70 d on test. The fact that XR behaved similar to NI and D200 steers for the initial 70 d on test indicate that the coated implant was not releasing exogenous hormones as intended by design.

From 71 to 140 d on test tabulated ADG was greatest for XR and D200 steers. Steers in the XS and XR treatment groups were not different from one another for ADG from 71 to 140 d. Additionally, improvements in gain efficiency and GED for XR from 71 to 140 d mirror those of D200. The fact that XR and D200 implants behaved so similarly during the initial 140 d on test and there after indicate that the additional coating applied to the XR implant was successful at delaying the release of anabolic constituents until at least d 70, in which improvements from 71 to 140 d for XR over negative control mirror those of the D200 implant group. It has been demonstrated in FOIA (2017) that for 70 d postimplantation steers receiving a Revalor-XR implant were not different from negative controls. However, from 71 to 200 d postimplantation cattle implanted with Revalor-XR had improved gain and gain efficiency over negative controls (FOIA, 2017). McLaughlin et al. (2013) reported that steers receiving a coated implant had a marked improvement for interim period ADG from d 75 to 140 over steers administered a noncoated implant.

From 141 to 213 d on test tabulated ADG was the greatest for XR and lowest for E200 steers. The XR and E200 steers received equal anabolic doses implanted at the exact same time. The only differences between the two implants was a polymer coating that is intended to delay release of anabolic constituents until at least 70 d postimplantation. This was the reason for implanting steers with a noncoated implant containing equal dose and ratio of TBA and E<sub>2</sub>, 213 (E200) and 143 (D200) d prior to harvest. Differences for XR and E200 steers from 141 to 213 d on trial are similar to results reported by McLaughlin et al. (2013) in which steers implanted with a coated implant had greater ADG and G:F over steers receiving a noncoated implant from d 140 to 200 postimplantation. Likewise, ADG from 141 to 213 d for XR was improved by 5% over D200 steers. Explant data for XR suggests changes in the ratio of TBA and  $E_2$  over time (Revalor-XR, FOIA, 2017). These changes in ratio of TBA and E<sub>2</sub> could explain minor improvements in ADG for XR over D200 from 141 to 213 d. The calculated GED was lowest for XR and greatest for XS while all other treatments were intermediate from d 141 to 213. The GED improvements for XR over XS could be due to differences in DMI above maintenance, the XS steers consumed greater DMI from 141 to 213 d, and GED is determined using (ER/d in Mcal/d) which is calculated on the basis of intake above maintenance.

Differences in gain performance between XR and E200 is similar to results in steers subjected to either coated or noncoated implants on the same day and fed to equal days on feed (McLaughlin et al., 2013). The relative gain response graph indicates that the effective payout of the XR implant was very similar to that of D200. Additionally, daily gain responses for steers in E200 demonstrated a very different payout period than XR or D200 steers. The differences in implant payout period demonstrate that the use of an XR implant is similar to D200 for gain performance and carcass responses. However, the fact that the XR was implanted at the exact same time as E200 steers, demonstrates that the coated implant indeed did delay the release of active compounds for at least 70 d as indicated by performance and sera metabolite responses. These data indicate that the polymer coating applied to the XR implant was successful in altering hormone payout to occur approximately 70 d following implantation.

# **Carcass Responses to Implant Treatment**

The use of implants improved HCW over NI steers. Steers in the XS treatment had the greatest HCW, which is to be expected when administered the greatest dose of E<sub>2</sub>; however, it is of interest

that steers in D200 had greater HCW than E200 steers. Presumably, the same implant should provide equal improvements in gain and HCW. This, however, was not the case in the present study. As previously mentioned, increased BW results in increased intake needed for maintenance requirements, which coupled with no exogenous source of hormone late in the feeding period, might decrease cattle performance and carcass weight in E200 compared to D200 steers (Johnson et al., 1996a; Parr et al. 2014). Steers from XR did not differ from E200 or D200 in HCW; altering anabolic release, or altering the time in which anabolic implants are offered can have tremendous impacts on overall value generated between implants with equal doses and ratios of anabolic hormones. Steers from the XS treatment had the greatest BF and dressing percentage, over all other treatments, which was likely a product of greater DMI throughout the course of the study. Statistically, there were no differences among treatments for LM area, although XR had a 5% increase in LM area over NI and E200 steers, and marbling was not different between the NI or XR steers. Marbling was also similar between XS and NI steers. Steers receiving a noncoated implant (i.e. E200 or D200) had the lowest marbling scores relative to NI steers. Smith et al. (2017) indicated that noncoated and coated implants differentially alter adipogenic gene expression in LM biopsies of steers. Smith et al. (2017) demonstrated that steers administered a noncoated implant containing 120 mg TBA + 24 mg E, had decreased expression of PPARy, GPR 41, and GPR 43, important genes involved in adipogenesis, in LM biopsies relative to negative controls or steers implanted with a Revalor-XS. Bryant et al. (2010) demonstrated that steers implanted with a noncoated implant containing 80 mg TBA + 16 mg  $E_2$  and re-implanted with a noncoated implant containing 120 mg TBA + 24 mg  $E_2$  on d 56 had decreased marbling scores relative to controls at equal empty body fat percentage. In the present study, both E200 and D200 steers exhibited increases in final live BW that would require elevated fat deposition to mitigate depressions in marbling score. Guiroy et al. (2002) noted that steers administered a noncoated implant containing 80 mg TBA + 16 mg  $E_2$  and re-implanted with a noncoated implant containing  $120 \text{ mg TBA} + 24 \text{ mg E}_2$  required a 42-kg increase in final shrunk BW to reach similar body composition of nonimplanted steers. Both E200 and D200 groups had equal empty body fat to NI steers, the fact that overall marbling score depression occurred warrants further investigation into the mechanisms

of how anabolic payout impacts marbling scores, even in the face of increased final live BW and equal EBF. The XS steers had the greatest calculated EBF % and were different from all other treatments. This increase in calculated EBF % is likely a product of increased HCW and subcutaneous BF, as well as an increase in marbling score associated with greater intakes and adiposity. While no differences were detected among treatments for yield or quality grade distribution, steers in the XR treatment had equal numbers of carcasses with a marbling score of 500 or higher as NI steers (50.0 vs. 50.0 %), whereas steers from E200 only had 9.1% of carcasses with a marbling score of 500 or higher.

# Sera Metabolite Responses to Implant Treatment

The implant  $\times$  day interaction for circulating E<sub>2</sub> concentrations detected in these steers indicates altered payout characteristics for the various implants as evidenced by implant treatments exhibiting differing values for E, in circulation across time. Implantation with a combination TBA and E, implant increased circulating E, concentrations over NI in the present study, which is consistent with others (Johnson et al., 1996a; Bryant et al., 2010; Blackwell et al., 2014; Parr et al., 2014). Assay sensitivity for  $E_2$  quantification in bovine sera was 0.5 pg/mL; values for circulating  $E_2$  concentrations in NI steers were similar to ovariectomized heifers (Day et al., 1984) and lower than values for nonimplanted steers reported previously (Johnson et al., 1996a; Blackwell et al., 2014; Parr et al., 2014), or in heifers (Bryant et al., 2010). These lower sera E<sub>2</sub> levels for the NI steers might be a function of enhanced assay sensitivity in the present study. Johnson et al. (1996a) reported that E<sub>2</sub> values in sera were greatest at d 21 postimplanting in steers given a combination implant that contained 120 mg TBA and 24 mg E<sub>2</sub> and that values declined after that, however, E, in sera remained elevated relative to negative controls throughout the entire study. Sera  $E_2$  levels for XS decreased from d 14 to 35 and increased from d 35 to d 70, which is due to external polymer degradation of the coated portion of the implant, and this secondary increase in circulating E, for XS has been reported previously (Blackwell et al., 2014; Parr et al., 2014). The steers in XR had similar values to NI and D200 until d 70 of the study for circulating E<sub>2</sub> concentrations, indicating that the polymer coating applied to the implant pellets did delay the release of anabolic constituents until 35 to 70 d postimplanting, with peak sera E, values occurring 105 d postimplantation. Both E200 and XS would have contained 20 mg of E, for E200 or 16 mg of E, for XS in the implant pellets during the initial 35 d period following study initiation; values for circulating levels of E, in sera from E200 steers was greater than XS and all others on d 35. It is interesting to note that between both altered release formulations (i.e. XR and XS), that maximum sera E<sub>2</sub> concentrations occurred at the same time (d 105). By d 105, the coated portion of XS was likely paying out as indicated by others (FOIA, 2007; Parr et al., 2011a; Parr et al., 2011b; Blackwell et al., 2014; Nichols et al., 2014; Parr et al., 2014). The peak in sera E<sub>2</sub> concentrations for XR and XS occurred at a much later time-point postimplantation relative to E200 or D200 steers. The coated portion of XS would have had greater amounts of E<sub>2</sub> in the implant pellets than XR in a different ratio of androgen to estrogen. It is also interesting to note the large differences in circulating  $E_{2}$  and 17 $\beta$ -TbOH levels at 35 d postimplanting for E200 (d 35) and D200 (d 105) steers in the present study. The lower sera concentrations of E<sub>2</sub> and  $17\beta$ -TbOH for E200 vs. D200 at 35 d postimplanting are difficult to elucidate; however, there is the potential that BW differentially alters payout of exogenous hormones from the implant pellets, and can subsequently alter peak sera levels of hormones at 35 d postimplantation. To further investigate these differences in sera E<sub>2</sub> and  $17\beta$ -TbOH at 35 d postimplanting, we looked at the ratio of sera concentrations of  $E_2$ , for D200 on d 105 and E200 on d 35 (same time-point relative to when the implant was administered) and this ratio was 2.48 to 1. Likewise, we evaluated the ratio of sera concentrations of  $17\beta$ -TbOH for D200 on d 105 and E200 on d 35 and this ratio was 2.77 to 1. These ratios of 2.48 to 1 and 2.77 to 1 for E, and  $17\beta$ -TbOH, respectively, at 35 d postimplanting are both very similar to one another, and indicate that payout of exogenous hormones from identical implants formulations are influenced by BW. Also, it is important to realize that steroid hormones are metabolized to less biologically active metabolites in the body in two distinct phases. During phase I metabolism, there is the addition of reactive groups that alter the parent metabolite by a wide variety of enzymes, several steroidal hormones are catalyzed by the hepatic enzyme cytochrome P450, and during phase II metabolism phase I metabolites are conjugated with charged or polar species to facilitate elimination from the body (Pozo et al. 2015). Differences in circulating steroid hormones in steers receiving identical noncoated implants at 213 (E200) or 143 (D200) d prior to harvest might be explained by inducible enzymatic activity

differences at timing of implantation, due to proximity of previous calf-hood implants. Also, BW at timing of payout and ratio of androgen to estrogen in implant formulations can have influences on circulating concentrations of  $E_2$  and 17 $\beta$ -TbOH, in sera at various time points postimplantation. It is also important to realize that levels of hormones in circulation are influenced by many things such as: release rate of exogenous hormones from implant pellets, half-life of these exogenous hormones in circulation, differences in steroid hormone binding proteins, and differences in phase I and II metabolism of steroid hormone (Blackwell et al., 2014; Parr et al., 2014; Pozo et al., 2015).

The implant  $\times$  day interaction for circulating IGF-I concentrations indicate that payout was altered for the various test implants as evidenced by implant treatments exhibiting differing values for IGF-I across days on feed. Implantation with TBA and E, increased circulating concentrations of sera IGF-I in the present study, which is consistent with others (Johnson et al., 1996b; Bryant et al., 2010; Parr et al., 2014). Bryant et al. (2010) reported increased sera IGF-I values in circulation by 42 d in heifers implanted with TBA and E<sub>2</sub>, whereas steers implanted with TBA and E, increased sera IGF-I in circulation by d 21 and 27, respectively (Johnson et al., 1996b; Parr et al., 2014). Reinhardt et al. (2013) reported no differences in circulating concentrations of plasma IGF-I in steers administered a coated implant containing [200 mg TBA + 40 mg  $E_2$  (total): 80 mg TBA + 16 mg  $E_2$  (noncoated) and 120 mg TBA + 24 mg  $E_2$  (coated)] relative to NI steers at 28 d postimplantation.

Elevated NEFA values are a measure of adipose tissue catabolism. Implanting with TBA and  $E_2$  did not impact circulating concentrations of NEFA in the present study which is consistent with (Parr et al., 2014) who detected no differences in circulating concentrations of NEFA in steers given no implant, noncoated implant, or a coated implant. Others have also reported similar results, when implanting heifers with TBA (Heitzman and Chan, 1974) or steers with hexoestrol, TBA, or in combination (Galbraith and Watson, 1978). In contrast, Bryant et al. (2010) reported increased sera concentrations of NEFA in heifers implanted with TBA alone or in combination with  $E_2$ .

As many others have demonstrated previously (Heitzman and Chan, 1974; Heitzman et al., 1977; Bryant et al., 2010; Parr et al., 2014), SUN concentrations were decreased by the use of anabolic implants in the present study. Steers from XS group had decreased SUN relative to D200 steers at 14 d postimplant. Decreased SUN as an indicator of anabolism for E200 steers and XS steers vs. others at 14 d following the initiation of the study indicate that the implants were indeed stimulating protein accretion, the differences in degree of SUN depression following implanting are likely explained by the different doses and ratios of TBA and E<sub>2</sub> between E200 and XS. The E200 steers had the lowest SUN values compared to others at d 35 following treatment initiation. By 70 d postimplant SUN values were similar for XR and E200 steers. The depression in SUN on d 70 for XR steers occurred at a time when increased circulating concentrations of sera IGF-I were detected, and is likely a function of initiation of anabolic payout from the XR implant. The bleed date was merely a snapshot in time, and although the decreased SUN for XR at d 70 does not match with performance responses from d 0 to 70, from 71 to 140 d XR steers had improved gains, gain efficiency, and reduced GED over others. The decreased SUN for XR on d 70 could be an indication that the polymer coating had begun to degrade, and subsequently decreased circulating concentrations of SUN as an indication of anabolism of lean tissue. Degradation of polymer coating and initiation of hormonal payout resulted in improved performance for XR over NI and E200 steers from 71 to 140 d.

The use of anabolic implants increased circulating concentrations of sera 17 $\beta$ -TbOH in the present study. The rapid increases in circulating concentrations of 17 $\beta$ -TbOH in sera for XS and E200 was similar to what others have reported previously (Johnson et al., 1996a; Blackwell et al., 2014; Parr et al., 2014). The increases in sera 17- $\beta$ TbOH across days on feed occurred in concert with improvements in gain, gain efficiency, and circulating concentrations of sera IGF-I.

# CONCLUSION

Regardless of anabolic dose, ratio of TBA to  $E_2$ , or timing of implant administration: daily gain, HCW, and sera concentrations of  $E_2$ , IGF-I, and 17 $\beta$ -TbOH were increased, and SUN decreased by the use of implants. The use of XS improved ADG and DMI over all other treatments. The use of all implants improved ADG, DMI, and G:F over NI steers. The relative gain responses indicate that the use of an XR implant is comparable to D200. Likewise, differences in relative gain response between XR and E200 steers indicates that the polymer coating applied to XR altered the effective payout period of the XR implant

when compared to E200. The polymer coating applied to the XR implant delayed release of anabolic constituents in a manner that mirrored interim performance and relative gain responses to steers implanted with a noncoated implant with the same anabolic dose 70 d later (i.e. D200) than steers implanted with XR. The use of noncoated implants (i.e. E200 and D200) resulted in reduced marbling scores relative to NI steers, and these decreases in marbling score occurred even in the face of equal EBF that would be expected to allow implanted steers to have the same degree of marbling as NI steers. There is potential that the altered release rate of TBA and E<sub>2</sub> associated with coated implants altered mRNA expression of adipogenic genes and improved marbling scores over D200 and E200 steers. Body weight at the timing of implant and the ratio of androgen to estrogen in the implant formulation might differentially alter payout of exogenous hormones from implant pellets and subsequently alter hormone levels in circulation.

# LITERATURE CITED

- Blackwell, B. R., T. R. Brown, P. R. Broadway, M. D. Buser, J. C. Brooks, B. J. Johnson, G. P. Cobb, and P. N. Smith. 2014. Characterization of trenbolone acetate and estradiol metabolite excretion profiles in implanted steers. Environ. Toxicol. Chem. 33:2850–2858. doi:10.1002/etc.2757
- Blackwell, B. R., M. D. Buser, B. J. Johnson, M. Baker, G. P. Cobb, and P. N. Smith. 2013. Analysis of veterinary growth promoters in airborne particulate matter by liquid chromatography-tandem mass spectrometry. Eval. Vet. Pharm. Behav. Environ. 1126:137–148.
- Bryant, T. C., T. E. Engle, M. L. Galyean, J. J. Wagner, J. D. Tatum, R. V. Anthony, and S. B. Laudert. 2010. Effects of ractopamine and trenbolone acetate implants with or without estradiol on growth performance, carcass characteristics, adipogenic enzyme activity, and blood metabolites in feedlot steers and heifers. J. Anim. Sci. 88:4102–4119. doi:10.2527/jas.2010-2901.
- Day, M. L., K. Imakawa, M. Garcia-Winder, D. D. Zalesky, B. D. Schanbacher, R. J. Kittok, and J. E. Kinder. 1984. Endocrine mechanisms of puberty in heifers: Estradiol negative feedback regulation of luteinizing hormone secretion. Biol. Reprod. 31:332–341.
- FOIA. 2017. NADA 141–445. Trenbolone acetate and estradiol extended-release implant Extended- and delayed-release implant, Beef steers and heifers fed in confinement for slaughter, for increased rate of weight gain and improved gain efficiency during 70 to 200 days after implantation in beef steers and heifers fed in confinement for slaughter. Intervet, Inc, Madison, NJ.
- Galbraith, H., and H. B. Watson. 1978. Performance, blood and carcase characteristics of finishing steers treated with trenbolone acetate and hexoestrol. Vet. Rec. 103:28–31.
- Guiroy, P. J., L. O. Tedeschi, D. G. Fox, and J. P. Hutcheson. 2002. The effects of implant strategy on finished body weight of beef cattle. J. Anim. Sci. 80:1791–1800.

- Heitzman, R. J., and K. H. Chan. 1974. Alterations in weight gain and levels of plasma metabolites, proteins, insulin and free fatty acids following implantation of an anabolic steroid in heifers. Br. Vet. J. 130:532–537.
- Heitzman, R. J., K. H. Chan, and I. C. Hart. 1977. Liveweight gains, blood levels of metabolites, proteins and hormones following implantation of anabolic agents in steers. Br. Vet. j. 133:62–70.
- Johnson, B. J., P. T. Anderson, J. C. Meiske, and W. R. Dayton. 1996a. Effect of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers. J. Anim. Sci. 74:363–371.
- Johnson, B. J., M. R. Hathaway, P. T. Anderson, J. C. Meiske, and W. R. Dayton. 1996b. Stimulation of circulating insulin-like growth factor I (IGF-I) and insulin-like growth factor binding proteins (IGFBP) due to administration of a combined trenbolone acetate and estradiol implant in feedlot cattle. J. Anim. Sci. 74:372–379.
- Kirby, C. J., M. F. Smith, D. H. Keisler, and M. C. Lucy. 1997. Follicular function in lactating dairy cows treated with sustained-release bovine somatotropin. J. Dairy Sci. 80:273–285. doi:10.3168/jds.S0022-0302(97)75935-6
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using sas procedures. J. Anim. Sci. 76:1216–1231.
- Mader, T. L. 1998. Implants. Vet. Clin. North Am. Food Anim. Pract. 14:279–290.
- McLaughlin, C. L., D. T. Bechtol, T. E. LAwerance, K. Lechtenberg, W. M. Moseley, F. L. Prouty, B. H. Vanimisetti, and A. R. Waite. 2013. Comparison of the effects of long acting Synovex One with Revalor-XS and Synovex Plus on growth performance and carcass quality in steers. Prof. Anim. Sci. 29:147–156.
- Nichols, C. A., G. E. Erickson, J. T. Vasconcelos, M. N. Streeter, B. D. Dicke, D. J. Jordan, R. J. Cooper, T. L. Scott, and T. J. Klopfenstein. 2014. Comparison of Revalor XS, a new single-dose implant, to a Revalor IS and Revalor S implant strategy in finishing steers. Prof. Anim. Sci. 30:51–55.

- NRC. 1996. Nutrient requirements of beef cattle. 7th ed. Natl. Acad. Press, Washington, DC.
- Parr, S. L., T. R. Brown, F. R. Ribeiro, K. Y. Chung, J. P. Hutcheson, B. R. Blackwell, P. N. Smith, and B. J. Johnson. 2014. Biological responses of beef steers to steroidal implants and zilpaterol hydrochloride. J. Anim. Sci. 92:3348–3363. doi:10.2527/jas.2013-7221
- Parr, S. L., K. Y. Chung, M. L. Galyean, J. P. Hutcheson, N. DiLorenzo, K. E. Hales, M. L. May, M. J. Quinn, D. R. Smith, and B. J. Johnson. 2011a. Performance of finishing beef steers in response to anabolic implant and zilpaterol hydrochloride supplementation. J. Anim. Sci. 89:560–570. doi:10.2527/jas.2010-3101
- Parr, S. L., K. Y. Chung, J. P. Hutcheson, W. T. Nichols, D. A. Yates, M. N. Streeter, R. S. Swingle, M. L. Galyean, and B. J. Johnson. 2011b. Dose and release pattern of anabolic implants affects growth of finishing beef steers across days on feed. J. Anim. Sci. 89:863–873. doi:10.2527/jas.2010-3447
- Pozo, O. J., M. Ibáñez, J. V. Sancho, J. Lahoz-Beneytez, M. Farré, E. Papaseit, R. de la Torre, and F. Hernández. 2015. Mass spectrometric evaluation of mephedrone in vivo human metabolism: identification of phase I and phase II metabolites, including a novel succinyl conjugate. Drug Metab. Dispos. 43:248–257. doi:10.1124/dmd.114.061416
- Reinhardt, C. 2007. Growth-promotant implants: Managing the tools. Vet. Clin. North Am. Food Anim. Pract. 23:309–319. doi:10.1016/j.cvfa.2007.03.004
- Reinhardt, C. D., T. L. Lee, D. U. Thomson, L. K. Mamedova, and B. J. Bradford. 2013. Restricted nutrient intake does not alter serum-mediated measures of implant response in cell culture. J. Anim. Sci. Biotechnol. 4:45. doi:10.1186/2049-1891-4-45.
- Smith, Z. K., K. Y. Chung, S. L. Parr, and B. J. Johnson. 2017. Anabolic payout of terminal implant alters adipogenic gene expression of the longissimus muscle in beef steers. J. Anim. Sci. 95:1197–1204. doi:10.2527/jas.2016.0630.
- USDA. 1997. United States Standards for Grades of Carcass Beef. Washington, DC: Agric. Market. Serv., USDA.